INDIANA UNIVERSITY
BLOOMINGTON, INDIANA

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Dr. Joshua Lederberg
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The University of Wisconsin
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Dear Josh:

I am enclosing comments on your manuscript about phase variation in Salmonella instead sending them to Jim Crow only. You probably knew that the manuscript had been sent to me for refereeing. In any case, whether you knew or not, I think I would rather discuss the matter directly with you than send comments to Crow and have him transmit them to you anonymously. You will see that I had very great difficulty with the most important part of the paper and I think it is quite necessary to modify it in such a way that readers will not suffer in the same way that I have in trying to test the validity of your statements and to understand your meanings as you go along in the text. Not until I got to the discussion was I really clear as to the nature of the evidence for your conclusion and the nature of your reasoning about it. This is the main difficulty which I think needs ironing out and I think, through the comments I have made on the earlier parts of the paper before I really understood your drift in the discussion, that you will see how the changes should be made.

I am really very much impressed with the importance of the analysis and feel that it is, of course, exceedingly important for us in our interpretation of the phase variation in Paramecium.

With very best regards to you and Esther,

Cordially yours,

Tracy

T. M. Sonneborn

TMS:jws

Page 3, Par. 1. What you say here is, in a general way, correct and intelligible, but I think that the situation could be made a little clearer by separating the various types into two groups, dealing first with one and then with the other, because they have different meanings. Thus, the i:b and 1.2:enx types really are the only ones that imply what you state about the two groups of genetic homologies although the sentence in which you discuss this suggests that all three of the missing types lead to this conclusion. I would therefore advise omitting reference to the absence of i:1.2 at this point and delay mentioning it until after you have discussed the homology groups and begin to discuss, as you do in the next paragraph, the phenomenon of transduction itself. Finally, at the end of the first paragraph, where you state the factors within each group are mutually interchangeable by transduction, it would seem to me good to add the complementary statement: "But factors within different groups are not interchangeable by transduction." Otherwise you emphasize the conclusion without giving due emphasis to the major fact on which the conclusion rests.

Now, when you come to the second sentence in the next paragraph, of course you would have to make a modification if you omitted mention of the i:1.2 class in the preceding paragraph. That would be easy. Simply say after the first sentence: "There is also a third class which fails to appear in the previous example, namely, i:1.2. The absence of this class indicates further, etc."

Page 6, Line 4. I am surprised that you permit yourself to be so categorical and absolute in this statement. I would admit that one would expect the relative masses of the cytoplasm in recipient and donor to suggest a disparity in the results, but I am quite unwilling to say as strongly as you do that the phase should be inherited entirely from the recipient in transduction. Nothing is known about the mass of determinative material in the cytoplasm and it may be selectively absorbed by the migratory virus. This all seems unlikely, but I think it points up that you should be a little bit less absolute in your statement here.

In this connection there is a probably crazy experiment that might be fun to run just in order to permit you to make a more positive statement about the matter. Suppose, for example, you were to expose cells in a given phase 1 to virus from a culture which was in phase 2 and had a phase 2 different from that of the recipient. Could you readily detect whether exposure of the recipients to the virus increased the frequency of recipient cells which transformed to phase 2 without showing the phase 2 specific antigen of the host? What I am driving at is to see whether the virus brings or can bring into the recipient something which changes its phase by a mechanism other than that of introducing a different allele for that phase. This, if it happened, would perhaps suggest that the phage can carry a cytoplasmic determinant of phase irrespective of the specific antigen produced in that phase.

Page 6. I don't agree with the whole line of argument presented on this page. What you are in effect saying is that if there is a cytoplasmic state system of determination, then no other system of determination should have any effect. I believe this is a fundamental misstatement of the situation. And I can support it by facts from Paramecium if you wish. To indicate the general nature of them, I can say this much. I have

noticed in certain crosses between different strains that the expected development of heterozygosity for the pre-existent phase in the descendants of each exconjugant sometimes does not take place. Instead, the phase changes to some other one. This can all be explained very readily on the basis of the phase-environment relations in the two strains that were crossed. The new heterozygous genotype may have a different reaction system to environment than the previous homozygote did. Factors other than the cytoplasmic state thus play a part in determining which phase is expressed. Of course this is in agreement with what we know about the role of environmental conditions in influencing the cytoplasmic state itself. The main point in all this, of course, is to emphasize that the existence of a cytoplasmic state system of determination does not necessarily mean that other factors are not involved in determining the phase. But, as you state the case here, the argument against the existence of a cytoplasmic state system is based solely upon the demonstration that something else can affect the issue. This seems to me a totally unjustified type of argument.

When I read the remainder of the discussion on this page for the first time, I was astonished at the way you interpreted the results. It did not become clear to me why you did this until I had read much further in your paper. I think that you have been unconsciously influenced in the discussion of this experiment by ideas based on later experiments. Either you should say that the interpretation is based partly on material to be presented later, or here you should strictly interpret the results in terms of this experiment alone. So far as this experiment itself is concerned, I see nothing in it which is opposed to the cytoplasmic state hypothesis. Since you have a mixed culture of recipient cells, it could very well be that those which show transduction of the $H_1$ phase were cells which were in the $H_1$ phase while those which show transduction of the $H_2$ phase were those which were in that phase. To be sure, this leaves you with the unanswered question of why experiments (a) and (b) should have given different results in the sense that one yields transduction of phase 1 only while the other yields transduction of cells in both phases. But this could be due to a secondary effect, namely, one which depends upon whether the donor antigen genes are in the active or inactive condition. That is, phase 2 can be transduced only if the corresponding gene is in the active phase, but phase 1 can be transduced whether it is in the inactive or active phase. This, then, is a separate matter and tells nothing about which of the recipient cells are being transduced. They still could be limited to the ones which were showing the phase corresponding to the one which appears transduced. The major comment, then, that I would make on the argument presented on page 6 is that an influence of the donor phase under the conditions of this experiment need have nothing to do with testing the validity of the cytoplasmic hypothesis, for the design of the experiment permits no decision as to the role of the phase of the recipient since both phases are present in the culture.

Page 7, Par. 2. You have switched designations here from S. heidelberg to SL 28 in such a way that the reader is confused unless he is thoroughly familiar with this material or refers back to your description of materials. It would be much easier if you used the same designation for this strain in both parts of the paragraph.

Page 7, last paragraph. I suppose what you say here is correct and justified, but it is not immediately apparent to the reader that your conclusion of the transducibility of an inactive b is verified. Might not the reader wonder whether the b transductions, even though the predominant ones, were derived from donor cells in an active b phase as a result of impurity in the donor culture?

I find your discussion of hypothetical expectations on page 10, and their tabulation in table 7, in part very difficult to understand. Particularly, I don't understand hypothesis 3 at all, nor do I fully grasp what you intend by hypothesis 2. In my opinion, this part of your paper is unduly condensed and puts the greatest tax on the reader's interpretation as to what you mean. It seems to me that without some expansion of this material and some explanation of table 7, the reader is bound to be to some extent in a fog about your hypotheses and your analysis of them. I, therefore, very strongly urge that this part of the paper be redone in more detail with more elaborate exposition.

I also find it difficult to understand how the observed results can all be reconciled with your favored hypothesis if the results are simply to be explained on the basis that an inactive $H_2$ gene remain inactive after transduction. How then do you explain the failure to recover dl:r2 or dl:r2 when dl:d2 is transduced into rl:r2 or the failure to get dl:r2 when dl:d2 is transduced into rl:r2?

In the third line from the bottom on page 10, should not 121 be 111?

Page 11. I am continually bothered in the pages leading up to this point by what seems to be a continual shift from demonstration of the role of the inactive gene in the donor to the role of the active phase in the recipient. Nowhere do you bring these two together into a complete picture of the situation, but, it seems to me, shift from one to the other to the utter confusion of the reader. Thus, here on page 11 you seem to be saying that the phase of the recipient is very important. On the preceding page or so you develop the idea that the activity or inactivity of the $H_2$ locus is important. One is left with a good degree of bewilderment as to whether these are two aspects of one interpretation or whether the reader is misunderstanding you in your apparent statements that both factors are important. I'm really not sure myself whether you mean to lay the entire burden of the case on the activity of the $H_2$ locus or whether you are willing to admit that the phase which is phenotypically expressed in the recipient is also important.

Page 12, second line from the bottom. I think both the meaning and the writing would be improved if "another" were changed to "other."

Now, at last, as I get to page 15 I begin to make sense out of what was said in the preceding pages. I think it's important for you to realize, though, how utterly confused the reader can be until he gets to page 15. Now your hypothesis begins to hang together and one understands what you mean by the hypothesis of activity vs. inactivity of the $H_2$ locus. It has

not been clearly set forth before in such a way that the data can be entirely reconciled with the view. Perhaps the reader should have foreseen this, but he didn't--at least in my case. And I think it is up to you either to present the hypothesis in the first place with sufficient clarity so that the reader can understand what you mean, or defer the whole discussion of the hypothesis until the discussion section. By putting the hypothesis in earlier in inadequate form, the reader is simply confused and the whole thing doesn't make sense until he gets to page 15 of your manuscript. I think some kind of reconciliation of these difficulties is very much needed to make the manuscript effective. I see now how some of the objections that I raised earlier can be met, but I don't think the reader can see that, or is likely to see it, until he gets to page 15 when the damage is already done so far as his disposition is concerned.

Bottom of page 19. It is quite true, of course, that the work on caryonidal determination of mating types has <u>recently</u> been bolstered by the work of Nanney and Caughey on Tetrahymena, but I think it is only fair to call your attention to the fact that the caryonidal determination of mating type and its relation to nuclear differentiation had been first discovered in Paramecium and has been emphasized by me repeatedly since I first found it in 1937. At first I tried to reconcile this with the possibility of chromosomal segregation during anlagen formation. But very early I obtained evidence against this possibility, and in a Growth Symposium (I believe in 1946) made a special point of the significance of mating type determination in Paramecium for developmental differentiation. I don't mean to say that this ought to be inserted into your paper but I think it is desirable at least for you to know that the idea and the work go back much further than the recent work on Tetrahymena.

The second point in your summary again fails to get across the force of your conclusion. I doubt whether any reader could grasp from this statement the reasonableness of your conclusion. It just doesn't seem to follow from the second sentence under this point. Can't you put in another sentence which will explain more fully the validity of the jump from the fact to the conclusion?

In Table 5 I have marked two places where an entry has been made as to the Gal status of the transduced clones. In these cases there were no transduced clones and therefore neither a plus nor minus under the Gal column seems to be justified.